The Advantage of Fully Digital NMR and Pulsed Field Gradients

J. Ralph

Introduction

NMR is the most revealing and diagnostic tool we have in cell wall structural research. Two significant advances have recently been made coupling advanced theory with new electronic and equipment designs.

The first is the ability to now make the entire NMR architecture digital. Up to this point, many of the steps involve analog signals with incipient limitations and constraints. Recent digital technology, now fully established and stable in commercial instrumentation, has allowed an enormous gain to be made over the now dated analog technology. In particular, absolutely square digital filtering, many orders of magnitude more sharply defined than was previously possible, has drastically reduced electronic artifacts and the folding in of signals and noise from outside the regions of interest. The consequent gain in sensitivity and selectivity (and the ability to perform a whole new class of NMR experiments) is substantial. It also provides spectra with absolutely flat baselines (or baseplanes in 2- and 3-dimensional experiments) improving the quantitative aspects and the ability to extract data from minor components near the noise level (for example, important cross-linking structures that are, without labeling under our current detectability level). Also, digital oversampling, via a technique similar to that used in CD players, extracts a further two-fold sensitivity gain. Note that the savings in time for an experiment goes as the square of the sensitivity ratio!

The second major advance is not as new but has not until quite recently been accessible to 'normal' NMR operations. That is the application of pulsed field gradients to NMR. The clever theoretical proposals for the benefits of applying huge, but finely controlled, ramped field gradients during an NMR experiment have now become so well implemented in modern NMR spectrometers that spectroscopists exploit them routinely. The results of the technology are spectacular. Because of the quantum mechanical properties of such pulses on nuclear spins, spectra acquired using gradients are virtually artifact free. Artifacts, particularly those produced by the dominant methoxyl groups in our samples, obscure and obfuscate the data of smaller components such as, for example, special cell wall cross-linking structures.

Methods

Long range ^{13}C—^{1}H correlation (HMBC) spectra of a uniformly ^{13}C-enriched ryegrass lignin were run on two NMR machines, our own AMX-360 and the National NMR Facilities DMX-750. On the 360, 300 mg of sample was dissolved in 0.24 ml of acetone-d_6 and run for 60 h to give the top spectrum in Fig. 1. On the 750 (which currently only has a 5 mm probe, 122 mg in 0.4 ml acetone-d_6 was run for 24 h to give the bottom spectrum.

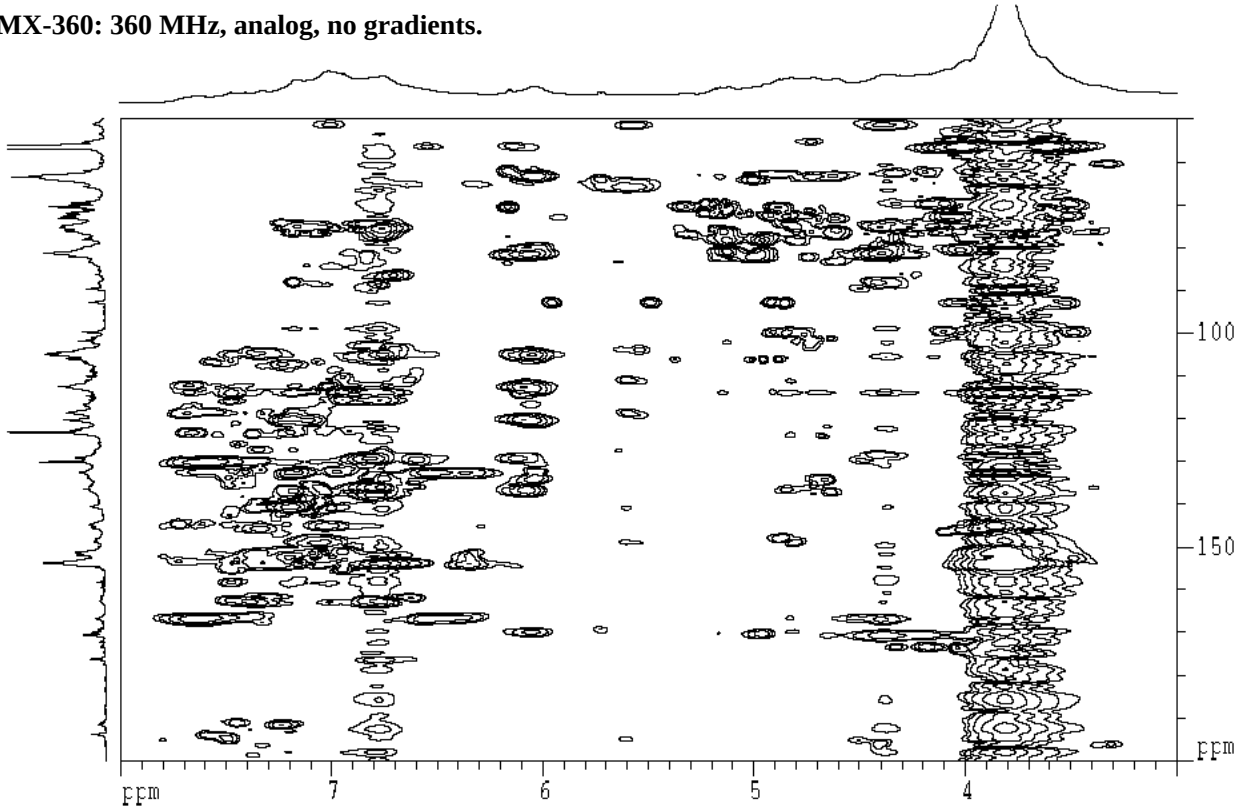
Results and Discussion

The differences in the spectra, Fig. 1, are spectacular. Ignoring the obvious dispersion gain from the higher field instrument, the freedom from T_1-noise artifacts in the digital/gradient system (boom spectrum) is striking. Peaks close to the methoxyl region are no longer ambiguous. In addition, the flat baseplanes and improved sensitivity allow us to look closer down to the noise level to reveal many more potentially valuable correlations. The only drawback is that we now have a great many more assignments to make in this spectrum!!

Conclusions

The Center's NMR instrumentation, has been a tremendous asset to the Cell Wall program. We have always been at the detectability limit, pushing the envelope of the technology to unravel the mysteries of the cell wall. The large gain in sensitivity via the digital system and oversampling, the ability to detect much closer to the noise level because of absolutely flat baselines/planes, and the tremendous artifact reductions available using gradients makes the updated system a must-have in this type of research.

AMX-360: 360 MHz, analog, no gradients.



DMX-750: 750 MHz, digital, gradients.

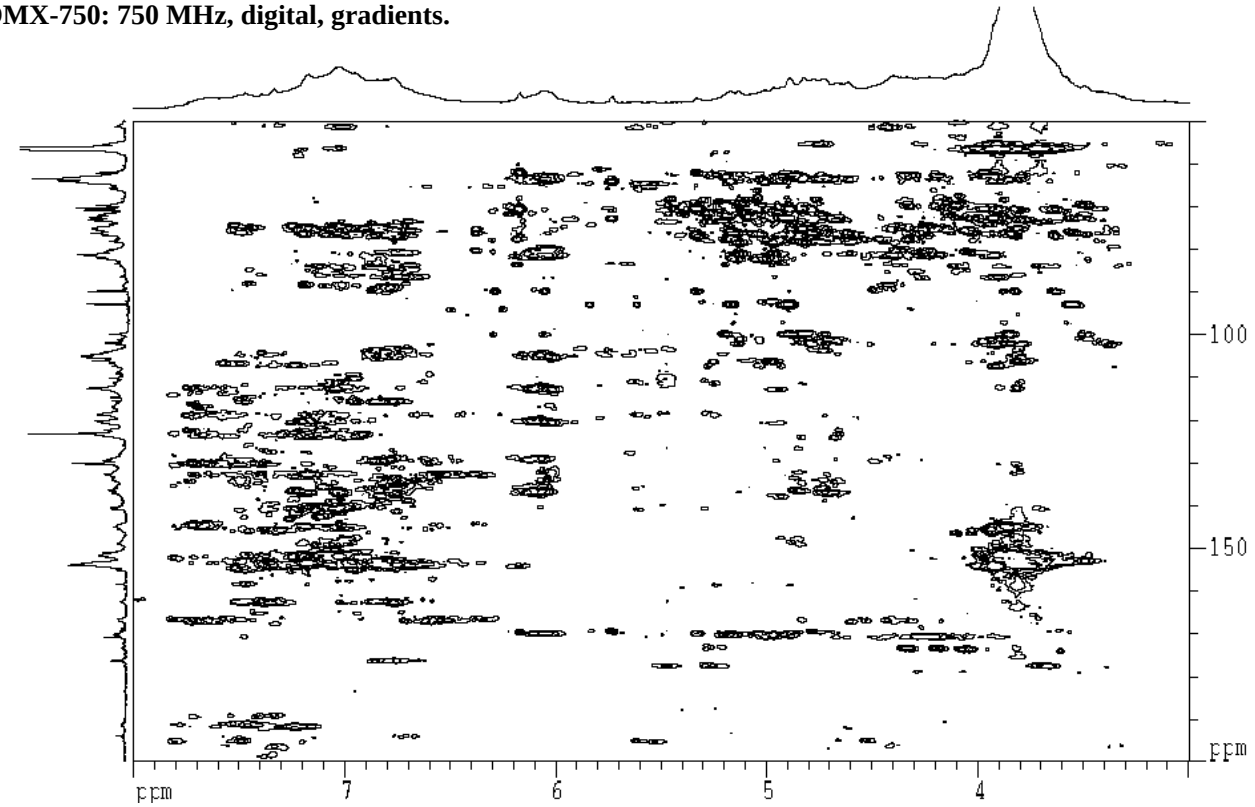


Figure 1. Comparison (unfair) of an HMBC spectrum of a ryegrass lignin with and without gradients and digital acquisition. Top spectrum: AMX-360, 300 mg, 60 h on our non-digital machine without gradients. Bottom spectrum: DMX-750, 122 mg, 24 h. Note particularly the absence of T_1-noise artifacts obfuscating the methoxyl region in the top spectrum (ca 4 ppm on the proton scale), the richness of detectable peaks due to the ability to approach the flat base-plane more closely (bottom spectrum), and the enhanced dispersion resulting from the MHz difference.